



HAL
open science

Gateway to genetic exchange? DNA double-strand breaks in the bdelloid rotifer *Adineta vaga* submitted to desiccation.

B Hespeels, K Knapen, D Hanot-Mambres, A-C Heuskin, F Pineux, S Lucas,
Romain Koszul, K van Doninck

► To cite this version:

B Hespeels, K Knapen, D Hanot-Mambres, A-C Heuskin, F Pineux, et al.. Gateway to genetic exchange? DNA double-strand breaks in the bdelloid rotifer *Adineta vaga* submitted to desiccation.. Journal of Evolutionary Biology, Wiley, 2014, pp.1334-45. 10.1111/jeb.12326 . pasteur-01420001

HAL Id: pasteur-01420001

<https://hal-pasteur.archives-ouvertes.fr/pasteur-01420001>

Submitted on 16 May 2017

HAL is a multi-disciplinary open access archive for the deposit and dissemination of scientific research documents, whether they are published or not. The documents may come from teaching and research institutions in France or abroad, or from public or private research centers.

L'archive ouverte pluridisciplinaire **HAL**, est destinée au dépôt et à la diffusion de documents scientifiques de niveau recherche, publiés ou non, émanant des établissements d'enseignement et de recherche français ou étrangers, des laboratoires publics ou privés.



Distributed under a Creative Commons Attribution - NonCommercial - ShareAlike| 4.0
International License

1 **SEX UNCOVERED SPECIAL ISSUE; Gateway to genetic exchange? DNA double-**
2 **strand breaks in the bdelloid rotifer *Adineta vaga* submitted to desiccation.**

3

4 Boris Hespeels^{1,2}, Manon Knapen¹, Delphine Hanot-Mambres^{2,3}, Anne-Catherine Heuskin^{2,4},
5 Florent Pineux^{5,6}, Stéphane Lucas^{2,4}, Romain Koszul^{5,6} and Karine Van Doninck^{1,2}.

6 1. University of Namur, Department of Biology, URBE, Laboratory of Evolutionary
7 Genetics and Ecology, 5000 Namur, Belgium.

8 2. Namur Research Institute for Life Sciences (NARILIS), 5000 Namur, Belgium.

9 3. University of Namur, Research Unit in Biology of Microorganisms (URBM), 5000
10 Namur, Belgium.

11 4. University of Namur, Research Centre for the Physics of Matter and Radiation (PMR),
12 5000 Namur, Belgium.

13 5. Department of Chemistry, University of Namur 5000 Namur (Belgium)

14 6. Namur Research College and Department of Chemistry, University of Namur, Namur,
15 Belgium

16 7. Institut Pasteur, Groupe Régulation Spatiale des Génomes, 75015 Paris, France

17 8. CNRS, UMR 3525, 75015 Paris, France

18

19 Running title: genome instability of desiccated bdelloids

20 Corresponding Authors:

21 karine.vandinck@unamur.be; Phone +32(0)81/72.44.07; Fax +32(0)81/72.43.62.

22 and

23 romain.koszul@pasteur.fr; Phone +33(0)140613325; Fax +33(0)185688960

24

25 **Abstract**

26 The bdelloid rotifer lineage *Adineta vaga* inhabits temporary habitats subjected to frequent
27 episodes of drought. The recently published draft sequence of the genome of *A. vaga* revealed
28 a peculiar genomic structure incompatible with meiosis and suggesting that DNA damage
29 induced by desiccation may have reshaped the genomic structure of these organisms.
30 However, the causative link between DNA damage and desiccation has never been proven to
31 date in rotifers. To test for the hypothesis that desiccation induces DNA double-strand breaks
32 (DSBs), we developed a protocol allowing a high survival rate of desiccated *A. vaga*. Using
33 pulsed-field gel electrophoresis to monitor genomic integrity, we followed the occurrence of
34 DSBs in dried bdelloids and observed an accumulation of these breaks with time spent in
35 dehydrated state. These DSBs are gradually repaired upon rehydration. Even when the
36 genome was entirely shattered into small DNA fragments by proton radiation, *A. vaga*
37 individuals were able to efficiently recover from desiccation and repair a large amount of
38 DSBs. Interestingly, when investigating the influence of UV-A and UV-B exposure on the
39 genomic integrity of desiccated bdelloids we observed that these natural radiations also
40 caused important DNA DSBs, suggesting that the genome is not protected during the
41 desiccated stage but that the repair mechanisms are extremely efficient in these intriguing
42 organisms.

43 **Keywords:** DNA damage; asexual reproduction; PFGE; UV radiation; DNA repair;
44 anhydrobiosis, horizontal gene transfer.

45 **Introduction**

46 First described by van Leeuwenhoek in 1702, bdelloid rotifers are microscopic invertebrates
47 that inhabit all types of freshwater environments, with a preference for temporary habitats that
48 dry out frequently. Since their discovery, no males, vestigial male structures or
49 hermaphrodites have ever been observed. Females exhibit a clonal mode of reproduction: they
50 produce eggs through mitotic divisions without apparent chromosome pairing nor reduction in
51 chromosome number (Hsu 1956a, 1956b). These observations brought the controversial
52 hypothesis that bdelloids have been reproducing purely asexually for more than 40 million
53 years (reviewed in Danchin et al. 2011). Recently, the draft sequence of the genome of the
54 bdelloid rotifer *Adineta vaga* yielded positive evidence of an ancient ameiotic evolution: the
55 genome of this species exhibits a peculiar structure in which allelic regions are massively
56 rearranged and sometimes found on the same chromosome in a palindromic fashion. No
57 homologous chromosomes are therefore present in this genome, ruling out the ability of *A.*
58 *vaga* to perform conventional meiosis (Flot et al., 2013). These various lines of evidence
59 converge on suggesting that bdelloids have been able to survive and diversify for millions of
60 years without sex, yielding more than 400 morphologically defined species (Segers 2007),
61 which makes them of particular interest to evolutionary biologists (Mark Welch and Meselson
62 2000).

63 Besides their notorious ancient asexuality, bdelloids are also known for their extreme
64 tolerance to desiccation. At any stage of their life cycle, they can enter a metabolically
65 quiescent state of anhydrobiosis for a prolonged period of time (Ricci et al., 2007). This
66 characteristic enables bdelloid rotifers to colonize limno-terrestrial habitats such as mosses and
67 lichens. Thriving in such ephemeral habitat is considered unusual because most animals and
68 plants die instantly if they experience complete desiccation. So far, only species belonging to
69 three animal phyla have been shown to be able to survive and reproduce in ephemeral

70 habitats: bdelloid rotifers, tardigrades and nematodes. Animals belonging to these clades are
71 therefore model organisms to unravel the mechanisms of desiccation tolerance within the
72 metazoans (Alpert, 2006). In addition, the asexual nature of bdelloid rotifers raises interesting
73 questions regarding a possible interplay between genetic exchange and desiccation (Flot et al.
74 2013).

75 The ability to withstand desiccation is a complex phenomenon that takes place at every level
76 of the anatomical and cellular organization. When bdelloids enter desiccation, their bodies
77 contract into a “tun” shape: the body becomes flattened dorso-ventrally and reduced in size,
78 the gut and vitellaria are packed ventrally while the trochi and foot are retracted inside the
79 body (Marotta et al., 2010). At cellular level, survival to desiccation requires adaptations that
80 maintain the function of macromolecules (*e.g.* proteins and DNA) and membranes despite
81 dehydration. Such result can be achieved through two non-exclusive ways: either by
82 preserving the integrity of these molecules, or by repairing them if they have been damaged
83 during the drying process. Sugars and antioxidants are key components that help maintaining
84 the integrity of proteins and membranes in all desiccated beings (Tunnacliffe et al., 2005;
85 França et al 2007). However, several studies have shown that genomic integrity is actually not
86 systematically preserved following desiccation. For instance, prolonged desiccation induces
87 DNA double strand breaks (DSBs) in bacteria such as *Deinococcus radiodurans* (Mattimore
88 & Battista 1996) as well as in tardigrades (Neumann et al. 2009) and chironomid larvae
89 (Gusev et al., 2010), which does not impede them to survive anhydrobiosis. A
90 counterexample is cyanobacteria, in which desiccation causes only limited damage to their
91 DNA (Shirkey et al., 2003, Billi 2009). In addition to desiccation resistance, *D. radiodurans*
92 also exhibits an extreme radiation resistance (Mattimore & Battista, 1996). These two
93 observations raised the hypothesis that the ability of *D. radiodurans* to survive both extreme
94 conditions was not coincidental, but linked to very efficient DSBs repair pathway(s)

95 (Zahradka et al., 2006). Interestingly, hydrated adults of the bdelloid rotifer species *A. vaga*
96 and *Philodina roseola* also exhibit extreme resistance to ionizing radiation (Gladyshev &
97 Meselson., 2008). They are able to reassemble into large DNA blocks a genome shattered into
98 pieces by multiple DSBs resulting from exposure to doses of more than 1000 Gy of gamma
99 radiation, and survive those damages (Gladyshev & Arhipova, 2010). By analogy with *D.*
100 *radiodurans*, it has been hypothesized that the extraordinary radiation resistance of bdelloid
101 rotifers was an adaptation to survive desiccation in their characteristic 'terrestrial' habitats
102 (Gladyshev & Meselson., 2008). However, no experimental evidence that desiccation causes
103 DNA DSBs in bdelloids has been obtained so far. Interest in this question was renewed when
104 the transcriptome of *A. ricciae* published by Boschetti et al. (2012) and the genome structure
105 of *A. vaga* (Flot et al., 2013) revealed high frequencies of gene conversion events and of
106 horizontal gene transfers (HGT), suggesting that the desiccation experienced by bdelloid
107 rotifers in their natural temporary habitat may have reshaped their genome over time. Notably,
108 the gene conversion inferred to occur between homologous regions, and the genomic
109 rearrangements, could be the result of DNA DSB repair through mitotic recombination
110 following desiccation. Also, during desiccation the integrity of membranes, including the gut
111 lining, could become compromised and therefore facilitate the passage of ingested foreign
112 DNA from the gut to the oocytes residing next to the digestive system. The exogenous DNA
113 can then be integrated into the germ-line DNA, either through homologous or ectopic
114 recombination during DNA repair, and be duplicated as suggested by Gladyshev & Arhipova
115 (2010). Such mechanisms occurring repeatedly over drought periods could account for the 8%
116 of genes that bear strong signatures of non-metazoan origin and are likely to originate from
117 HGT (Flot et al., 2013).

118 In order to tackle these intriguing hypotheses, we documented the genomic integrity of *A.*
119 *vaga* individuals submitted to prolonged desiccation. We first ruled out that DNA DSBs occur

120 only in dead animals, as well as the possibility that animals accumulating DSBs were not able
121 to recover upon rehydration and were therefore promised to a certain death. Knowing this, we
122 demonstrate for the first time that desiccation does induce DNA DSBs in these metazoans,
123 and that these damages are repaired after rehydration. We also studied the effect of UV-A and
124 UV-B radiation on genome integrity of desiccated *A. vaga* individuals, in order to look at the
125 influence of natural conditions on dried animals, and revealed an increase of DNA breaks
126 with UV exposure.

127

128 **Material and methods**

129 The question asked (whether bdelloid rotifers accumulate DNA DSBs during desiccation
130 without reducing their survival significantly) necessitates showing that i) DSBs accumulate
131 during desiccation, ii) DSB accumulation take place in live animals and not only dead ones,
132 iii) live animals with DSBs within their genome can survive desiccation upon rehydration and
133 that survival was not simply limited to animals that did not experience damage, iiiii) upon
134 rehydration DNA DSBs are repaired. To answer these points, we: i) used Pulsed Field Gel
135 Electrophoresis (PFGE) to measure the integrity of the genome during desiccation, ii) then
136 measured and compared kinetics of DSB apparition in a population of live and dead
137 desiccated animals, iii) genomes of all animals of a desiccated population were shattered into
138 small fragments using proton irradiation and survival rates were measured iiiii) and finally
139 repair kinetics were conducted. Below the methods used to reach these goals are outlined.

140 *Bdelloid rotifer culture*

141 All experiments were performed using isogenic *Adineta vaga* clones descending from a single
142 individual from the Meselson Laboratory (Van Doninck et al., 2009). The cultures were
143 maintained hydrated in 150 x 20 mm Petri dishes supplemented with natural spring water

144 (Spa®), at 16°C, and fed with *E. coli* 1655MG. To avoid bacterial proliferation and dust
145 accumulation, water was changed twice a month.

146 *Desiccation and survival rates*

147 Dense cultures of *A. vaga* individuals were starved for two days and then washed with Spa®
148 water to remove debris and dead animals. Individuals were detached from the bottom of the
149 Petri dishes using cell scrapers, collected in a 15 mL Falcon tube and centrifuged for 15 min
150 at 5000 rpm. The supernatant was removed and individuals were pooled and resuspended in
151 filtered Spa® water. 250 µl of this dense suspension, containing 10.000 to 15.000 *A. vaga*
152 individuals, were deposited in the center of a 3% Low Melting Point (LMP) agarose plate and
153 complemented with 1mL Spa® water. These Petri dishes were placed without lid in a climatic
154 WEKK 0028 chamber (Voetsch). The following desiccation conditions were applied: linear
155 decrease of relative humidity from 70% to 55% in 17 hours, linear decrease of relative
156 humidity from 55% to 41% during 1 hour, followed by 19 hours at 41% relative humidity.
157 After these 37 hours all the water had evaporated, the LMP layer was dry and the bdelloids
158 appeared desiccated. Dehydrated *A. vaga* individuals were maintained at 41% RH and 23°C
159 during 1, 7, 14, 21, 42 and 84 days of desiccation. Four desiccation plates were used for each
160 time point (1 for PFGE analysis and 3 plates measuring survival rates).

161 To determine the survival rate after desiccation, Petri dishes were removed from the climatic
162 chamber at specific intervals (see below) and rehydrated with 15 ml Spa® water. The survival
163 rate after desiccation was estimated by counting living and dead individuals 24 hours after
164 rehydration. Bdelloids were considered alive when the mastax moved in contracted
165 individuals or if they had fully recovered motility.

166 Group effect on survival rate was tested by varying the amount of *A. vaga* individuals (1, 10,
167 50, 100, 500, 1000 or 5000 individuals per plate) in the desiccation protocol while

168 maintaining the final volume at 1.25 mL water. These plates were transferred to a non-
169 hermetic room at 20°C where relative humidity was maintained around 40% (+/- 12%) using
170 a Frigor FDH 12 dehumidicator. Desiccated plates were kept dry during 14 days in this
171 specific experiment.

172 *Measuring the residual moisture content.*

173 In order to confirm complete desiccation of *A. vaga* clusters, the water content of hydrated
174 and desiccated (1 and 7 days) *A. vaga* clusters was determined using a thermogravimetric
175 analysis (TGA) following an adapted protocol from Alcazar et al. (2000). Each tested
176 condition was done in duplicate. TGA measures the amount of weight change of a material in
177 function of a temperature gradient and therefore detects residual water of dried samples.
178 Desiccated *A. vaga* individuals were scraped from their LMP agarose support with a surgical
179 blade. Hydrated specimens were detached from the bottom of the Petri dishes using cell
180 scrapers, collected in a Falcon tube, centrifuged 15 min at 5000 rpm, re-suspended in 2 ml
181 distilled water and centrifuged 3 times in order to remove impurities in the supernatant and all
182 excess water. Desiccated and hydrated samples were placed in a measuring pan of a
183 thermogravimetric analyser (Perkin-Elmer TGA 4000). The initial weight of the sample was
184 determined, then under a constant nitrogen flow of 55 ml/min, starting at 30°C with a heating
185 rate of 10°C/min, the temperature was raised to 60°C and maintained there for 100 min. The
186 temperature was then raised to 125°C and maintained for 7 min in order to complete the water
187 elimination and determine the weight or dry mass of the sample (free water residual mass).
188 Finally, temperature was raised to 600°C with a heating rate of 5°C/min to examine thermal
189 decomposition of the sample.

190 *Genomic DNA integrity*

191 Genomic DNA integrity was assessed using PFGE. Petri dishes were removed from the
192 climatic chamber at specific intervals (see below) and rehydrated with a cold solution of 50
193 mM EDTA 10 mM Tris (pH 8.0). One thousand contracted, desiccated *A. vaga* individuals
194 were harvested and resuspended in 25 μ L of cold 50 mM EDTA 10 mM Tris (pH 8.0), then
195 mixed with 25 μ L of 1% low melting point agarose (LMPA; NuSieve GTG) freshly melted in
196 a buffer containing 200mM EDTA, 100 mM Tris (pH 8.0) and finally casted in a plug mold at
197 42°C. After 15 min of polymerization, plugs were individually transferred into 500 μ L
198 digestion buffer (100mM EDTA 50mM Tris, pH8, supplemented with 1mg/mL proteinase K
199 (Fermentas) and 1% N-Lauroylsarcosine sodium solution), kept for one hour at 4°C and then
200 incubated 18 h at 56°C. As a control, we used plugs containing *S. cerevisiae* chromosomes
201 (BioRad) treated with the same digestion buffer. Plugs were then incubated in 1mL 0.5X TBE
202 at 4°C for 3h, rinsed with 0.5X TBE then kept in 1mL EDTA 0.5M (pH 8.0) at 4°C until use.
203 The complete lysis of the bdelloid individuals embedded within the plugs was controlled
204 visually with a microscope. Plugs were loaded in a gel (0.8% LMPA, 0.5X TBE) and
205 migration was performed using a BioRad CHEF-DR II instrument (14°C; 5.5V/cm, switch
206 angle of 120° and switch times of 60-185 sec for 22h with a linear ramp). The PFGE gel was
207 labeled in SYBR Gold (Invitrogen) and scanned with a BioRad Chemidoc XRS camera.
208 ImageLab 3.0 quantification software (SybrGold settings) and ImageJ were used to process
209 the images. Intact or slightly degraded chromosomes are expected to remain in the well, given
210 the size of the *A. vaga* genome being ~ 244 Mb for 12 chromosomes (Flot et al., 2013; Mark
211 Welch JL et al., 2004). DNA segments migrating within the resolution size of the PFGE
212 (~<2,2 kb) are therefore resulting from important chromosomal degradation.

213 *Killing A. vaga individuals*

214 In order to follow DNA degradation in *A. vaga* individuals killed before entering into
215 desiccation, the animals were desiccated following the same desiccation protocol but with

216 1.25 mL Spa® water supplemented with neomycin (Sigma) to a final concentration of 350
217 µg/mL. This dose is lethal to *A. vaga* and all individuals died right before complete
218 desiccation. Both the dead and living *A. vaga* individuals were maintained during 1, 21 and 42
219 days in the same dried conditions.

220 *Proton irradiation of desiccated A. vaga*

221 *A. vaga* individuals desiccated for one day were submitted to 1.7 MeV proton radiation
222 (delivering 25keV/µm) using Tandetron 2 MV (Wera et al. 2008; 2011). Dose rate was equal
223 to 10 Gy/min. Survival rate after radiation was investigated as for the desiccation protocol.
224 Number of DNA DSBs induced by proton radiation was calculated based on *A. vaga* genome
225 size, 240 Mb (Flot et al., 2013), and average molecular size observed after doses of 500 Gy
226 and 800 Gy.

227 *DNA repair kinetic*

228 Two repair kinetics were performed on *A. vaga*. First, we studied the DNA repair of DSB
229 damage induced by 21 days of desiccation. Second, *A. vaga* individuals desiccated for one
230 day were exposed to 800 Gy of proton radiation and DNA repair was investigated. In both
231 experiments, genomic integrity was checked on surviving individuals after 0, 2, 8, 24 and 48 h
232 of rehydration. PFGE setup was as described above.

233

234 *UV-A and UV-B radiations*

235 *A. vaga* individuals desiccated for one day were submitted to UV-B radiation (wavelength of
236 312 nm) generated by three TL 20 W/01 lamps (Philips, Eindhoven) placed 30 cm above the
237 rotifer individuals. The precise dose of radiation emitted was measured using a UVR
238 radiometer Vilber VLX3W with UV-B sensor (312 nm). The same protocol was performed

239 for UV-A radiation using three Philips 40W Cleo Performance lamps emitting at broad range
240 of UV-A (310-400nm with maximum intensity at 360 nm). Radiations were recorded using
241 the Vilber VLX3W radiometer with UV-A captor (360 nm). Covering the petri dishes with
242 dark paper blocking 100% of the UV-A and UV-B radiations assessed a potential heat effect
243 on survival rates.

244

245 **Results**

246 *Survival rates after desiccation are strongly influenced by “cluster formation”*

247 To address the impact of desiccation on the survival of *A. vaga*, we measured the survival rate
248 of various numbers of *A. vaga* individuals after 14 days of desiccation. The rate appeared
249 strongly dependent on the number of bdelloid individuals present in the drop of water
250 deposited on the desiccation medium (Figure 1a): while one isolated individual died 100% of
251 the time, batches of 5,000 individuals presented a ~ 93% survival rate. In this latter condition,
252 dead individuals were isolated on the plate, whereas living *A. vaga* individuals emerged from
253 clusters. Therefore, increasing the number of bdelloids involved in a desiccation experiment
254 enhances both the probability to form dense clusters and the survival rate. This observation
255 suggests that upon slow desiccation bdelloid rotifers tend to group and form clusters as a
256 natural strategy to escape death. In controlled conditions set to 23°C, 41% +/-1% RH, clusters
257 formed by 10.000 to 15.000 *A. vaga* individuals exhibited a survival rate at 14 days of 98%
258 (Fig. 1b), and still as high as 75% after 84 days of desiccation. For subsequent desiccation
259 experiments and survival rate evaluation, only *A. vaga* individuals present in desiccated
260 clusters with a minimum of 10.000 individuals were considered. A thermogravimetric
261 analysis was performed on dried *A. vaga* clusters to confirm complete desiccation of the
262 whole cluster. Water content of hydrated *A. vaga* rotifers was estimated at 94% and decreased

263 to a maximum residual water content of 6,5% of dry weight in 1 and 7 days desiccated *A.*
264 *vaga* clusters (supp. data 1) as observed by Lapinski & Tunnacliffe (2003) for dried bdelloids.

265 *DNA breaks accumulate during desiccation*

266 The integrity of chromosomes during desiccation was investigated through PFGE. First, in
267 order to test for the influence of the experimental procedure on the generation of DNA DSBs,
268 we treated chromosomes of *S. cerevisiae* embedded in agarose plugs similarly to the plugs
269 containing rotifers. No sign of degradation was observed, suggesting that the lysis *per se* does
270 not generate DNA DSBs (Fig. 2a, lane 9). The genome of *A. vaga* comprises 12 chromosomes
271 of ~20kb, and therefore *A. vaga* full-length chromosomes do not match the resolution of the
272 PFGE (between 225 to 2200 kb) and will remain in the wells. As expected, the control
273 genomic DNA of 1000 hydrated *A. vaga* individuals was observed to remain in the gel plug,
274 with a weak signal around 2 Mb (Fig. 2a, 2b, 2c, lane 2). No DNA smear was detected in *A.*
275 *vaga* individuals desiccated for one day, although a slight increase of DNA fragments > 2 Mb
276 was observed (Fig. 2a, lane 3). After 7 days of desiccation a well-defined smear appeared,
277 reflecting the apparition and migration into the gel of DNA fragments ranging in size between
278 225 and 2,200 kb (Fig. 2a, lane 4). The amount of chromosomal fragments migrating into the
279 gel kept increasing as a function of the time spent in desiccated state (Fig. 2a). Therefore,
280 although the drying process *per se* does not appear to generate significant amount of DSBs
281 (see lane 3 on Fig 2a: 1 day completely desiccated), a prolonged period in desiccated state
282 results in the accumulation of DNA DSBs in the bdelloid rotifer *A. vaga*.

283 Together, these two experiments also reveal that the influence of “group” formation onto
284 survival rate is not directly linked to protection against DNA DSB and/or increased repair
285 efficiency.

286 *Survival to desiccation is not related to genome integrity*

287 DNA DSBs were observed after 7 days or more in desiccated state (Fig. 2a) while the survival
288 rate remained high (>75%; Fig. 1b). The apparition of degraded DNA in our PFGE (Figure
289 2a) could eventually solely reflect the accumulation of DSBs in the genomes of the few dead
290 animals present in the cluster and not in those of living rotifers. To address this issue, we first
291 tested whether an increase in DNA damages was observed in 1,000 dead *A. vaga* individuals
292 submitted to desiccation, as compared to living ones. Death was induced by a lethal dose of
293 neomycin just before entering desiccation. If DNA DSBs occurred because of “death”, we
294 expected to observe a significant, precocious increase in DSBs in the dead group compared to
295 the control, since both were composed of the same number of individuals. After 21 or 42 days
296 of desiccation, the accumulation of DNA DSBs in the group of living individuals (with < 15%
297 mortality) was similar, both in timing and amount, to the one in the group of dead individuals
298 (Fig. 2b). This result confirmed that DNA DSBs accumulate during desiccation independently
299 of the animal condition (dead or “alive”). The accumulation of DNA DSBs observed on Fig.
300 2a is therefore the consequence of desiccation and not of mortality.

301 Then, we investigated whether the survival of desiccated *A. vaga* individuals was dependent
302 on the amount of DSBs present in the genome. To do so, we measured the survival rate of
303 one-day desiccated *A. vaga* individuals exposed to high dosage of ionizing radiation (proton
304 radiation, doses from 0 Gy to 800 Gy; Fig. 1c and 2c; M&M). Proton radiations generate
305 DSBs through direct interaction with the DNA molecule, in opposition to gamma radiations
306 that induce damages indirectly through the generation of reactive oxygen species from water
307 molecules (Gusev et al., 2010; S. Lucas pers comm). Proton radiations are therefore well-
308 suited here to induce breaks in dried rotifers. Fifty Gy of proton radiations was sufficient to
309 induce DSBs and DNA fragments clearly visible on the gel (Fig. 2c, lane 4). With average
310 fragment sizes of 450 and 365 kb for respective exposition to 500 Gy and 800 Gy (Fig. 2c,
311 lanes 7 and 8) an estimation of 0,004 DSB Gy⁻¹ MB⁻¹ is calculated (based on genome size of

312 244 Mbp; M&M). Despite these highly fragmented genomes in desiccated state, the survival
313 rate of *A. vaga* was not affected and reached 99% (Fig. 1c), a rate similar to non-irradiated *A.*
314 *vaga* individuals desiccated for one day (Fig. 1b). This result demonstrated that the
315 accumulation of DNA DSBs in desiccated *A. vaga* individuals does not alter significantly
316 survival rate, and that *A. vaga* are able to recover from massive DNA damages upon its exit
317 from the desiccated state.

318 *DNA Repair kinetic*

319 To investigate whether the genomes of dried *A. vaga* are efficiently repaired at the exit of the
320 desiccation process, we analyzed the DNA repair kinetics of individuals desiccated for 21
321 days and subsequently rehydrated for 48 hours. Restoration of complete mobility of
322 rehydrated *A. vaga* was observed between 2 to 24 h after rehydration. As expected, DNA
323 damages were observed in animals just rehydrated (Fig. 3a, lane 3). Then, the smear of small
324 DNA fragments ($< 2,200$ kb) progressively vanishes during the 48 hours spent in the
325 rehydrated state (Figure 3a). Similarly, the amount of large ($\geq 2,200$ kb) DNA segments also
326 gradually decrease in the gel, revealing the presence of an active DNA DSB repair mechanism
327 after desiccation. Interestingly, after 48 h of rehydration, large DNA fragments (≥ 2 Mb)
328 remained present in the genomes of fully mobile individuals.

329 We then tested further the ability of dried individuals to cope with massive DNA DSBs by
330 recording DNA repair of rehydrated individuals that were one-day desiccated and submitted
331 to 800 Gy of proton radiations. Interestingly, rehydrated *A. vaga* were able to repair these
332 damages (Figure 3b): they gradually reduced the amount of small DNA fragments (225 -1125
333 kb) of their shattered genomes and regained larger DNA fragments over time. Despite full
334 recovery of mobility 8h after rehydration, DNA damages were still observed in 48h
335 rehydrated *A. vaga*.

336 Overall, these results demonstrate that rehydrated *A. vaga* individuals submitted to desiccation
337 are able to handle extreme numbers of DNA DSBs, and underlie the presence of a remarkably
338 efficient DSB repair mechanism. In addition, we also show that the exit of the desiccated state
339 is not impaired by the presence of DNA breaks, and that these animals recover motility and
340 metabolic activity.

341 *Effect of UV-radiations on survival and genome integrity*

342 UV-A and UV-B radiation, in contrary to proton and gamma radiation, are radiations
343 commonly encountered in natural habitats. Whether such wavelengths are likely to affect the
344 survival and the genome integrity of dried bdelloids remains unknown. We asked whether the
345 combined effect of desiccation with UV exposure (other than the harsh proton irradiation
346 performed in laboratory conditions) would also have an effect on the amount of DNA DSBs.
347 To test this, *A. vaga* individuals desiccated for one day were exposed to increasing doses of
348 ultraviolet radiation (50, 100 and 150 kJ.m⁻²). Surprisingly, exposure to UV-A or
349 monochromatic UV-B radiations decreased the survival rate on the contrary to proton
350 radiations (see Fig. 1d). UV-B appeared systematically more harmful than UV-A: 10% of
351 dried *A. vaga* survived a dose of 150 kJ UV-B while 38% survived the same dose of UV-A
352 (Fig. 1d). Both UV-A and UV-B induced DNA damage: a smear was observed at each tested
353 dose on PFGE (Fig. 4). UV-B induced a higher amount of damage than UV-A with fragments
354 above 2 Mb still being observed at the highest UV-A dose of 150 kJ.m⁻². An increase in DNA
355 degradation was observed for both UV-A and UV-B following a dose-dependent curve (Fig.
356 4a and 4b respectively). In comparison with UV-treated desiccated individuals, no DNA
357 damage was observed for those protected from UV rays but experiencing the heating effect of
358 the UV lamp (Fig. 4, lanes 5, 7 and 9 on both gels). Overall, these results suggest that UV is
359 an important source of DNA DSBs in desiccated animals, and that the damages generated by
360 this type of radiations are more harmful to *A. vaga* rotifers than proton radiation. Seeking

361 protection from these electromagnetic radiations is therefore crucial for rotifers entering the
362 desiccation stage.

363

364 **Discussion**

365 *Desiccation in the bdelloid rotifer Adineta vaga*

366 In the present study, we refined the experimental conditions needed to promote desiccation of
367 adult bdelloid rotifers with optimal survival (i.e. through a controlled, progressive evaporation
368 of water over 37 hours on a LMP substrate). Interestingly, we observed that the ability to
369 survive desiccation was highly correlated with the bdelloids aggregating together. This
370 aggregation phenomenon had already been observed in desiccation-resistant tardigrades and
371 nematodes, and was described as a way to reduce the evaporation rate by reducing exposed
372 body surface area (Ivarsson 2004). Clusters of *A. vaga* individuals were already present 15h
373 through the dehydration process, when agarose was still hydrated. Given that dead individuals
374 did not reorganize into clusters, such aggregation appears as a dynamic response of *A. vaga*
375 individuals upon increasing hydric stress. After 37h of slow dehydration, LMP agarose with
376 clustered *A. vaga* individuals were completely dry as determined by the thermogravimetric
377 analysis. According to the literature, complete desiccation is reached when water content
378 decreases below 10% of dried mass. At this level there is no longer enough water to form a
379 monolayer around macromolecules, preventing enzymatic reactions and therefore metabolism
380 (Billi and Potts, 2002; Alpert 2005).

381 In the nineteenth century, Davis (1873) was the first scientist to study the desiccation
382 mechanism in bdelloid rotifers. He proposed that in addition to body contraction, the
383 gelatinous fluid secreted around the body of bdelloid rotifers is a key factor for desiccation
384 survival. Clusters of desiccated bdelloids therefore appear to be capped in a “gelatinous

385 varnish” which may enhance protection. The nature of this substance is still unknown and it
386 remains an open question, as well as the influence of the clustering on the ability of bdelloid
387 rotifers to resist environmental stresses in general.

388 *Impact of desiccation on genomic integrity*

389 Although the causality between desiccation and resistance to radiation-induced DNA damages
390 in bdelloid rotifers has been regularly invoked, it was never demonstrated experimentally
391 (Gladyshev& Meselson, 2008). Here we show that indeed the genome of dried *A. vaga*
392 individuals is accumulating DNA DSBs as a function of time spent in desiccated state. The
393 genomic integrity was equally impacted in living and dead dried *A. vaga* individuals,
394 suggesting that DNA repair is not an active process during desiccation and that cells of *A.*
395 *vaga* experiment a suspended metabolic state.

396 Entering a desiccated state is generally accompanied by a metabolic shutdown and the
397 generation of reactive oxygen species (ROS) known to promote DNA damage if not
398 neutralized by antioxidants (França et al 2007, Gusev et al 2010). Interestingly, recent data
399 suggest that strong antioxidant machineries appear to be present in *A. vaga* protecting its
400 cellular components from oxidative damage during exposure to high doses of ionizing
401 radiation (Krisko et al., 2012). This was corroborated by genomic data indicating that gene
402 families involved in resistance to oxidation have significantly expanded in this lineage (Flot et
403 al., 2013). Such an arsenal of antioxidants may have been selected over time to protect
404 proteins from oxidative damage during desiccation, including those involved in DNA DSB
405 repair.

406 *Effect of DSB accumulation on genomic content*

407 We show that the survival rate is equivalent (and almost 100%) for non-irradiated one-day
408 desiccated bdelloids and dried animals submitted to 800 Gy proton radiations (carrying more

409 than 700 DNA DSBs per cell). Our results confirm the extraordinary radiation resistance of
410 dried *A. vaga*, as observed by Gladyshev et al. (2008) on hydrated individuals. Proton
411 radiations are targeting all tissues without discrimination; it is therefore very unlikely that the
412 observed DNA DSBs in desiccated *A. vaga* are restricted to somatic cells only. A previous
413 study by Gladyshev & Arkhipova (2010) demonstrated that DNA damage induced by gamma
414 radiation upon multiple *A. vaga* generations resulted in the loss of a mariner-like *AvmarI*
415 transposon copy. This result confirmed the presence of DNA DSB damage in oocytes that are
416 subsequently repaired and are potentially responsible of the elimination of transposable
417 elements. Indeed, during homologous recombination repair, ectopic crossing over between
418 dispersed transposable elements can result in lethal chromosomal rearrangements, selecting
419 against a high TE content (Gladyshev & Arkhipova, 2010). By analogy with these
420 observations, desiccation cycles observed in the natural environment may be the source of
421 DNA DSBs needed to purge TEs from this genome unable to undergo conventional meiosis
422 (Flot et al. 2013). Indeed the genome of *A. vaga* contains only about 3% of transposable
423 elements, which is much less than the percentage found in other metazoans (Flot et al., 2013).
424 Besides the DNA DSBs induced by desiccation, RNA-mediated silencing machineries may
425 contribute to the prevention of TE expansion in *A. vaga* (see Flot et al., 2013).

426 *Recovery from desiccation: DNA repair kinetic*

427 The massive amount of DNA damage induced by high doses of radiation (Fig. 2c) is not
428 preventing the recovery process of *A. vaga*, neither in hydrated (Gladyshev et al., 2008) nor in
429 desiccated state. Our rehydration kinetics shows that full mobility recovery is reached after 8h
430 of rehydration despite the presence of DNA damages and that DNA repair is active upon
431 rehydration since we observe a decrease in small DNA fragments. At 48h DSBs were still
432 present but less. This is consistent with data collected in the chironomid species *P. verplanki*
433 where the genome integrity was retrieved only after 96h of repair (Gusev 2010). Fisher et al.

434 (2013) also found that the repair of cyclobutane-pyrimidine dimers (CPDs) induced by UV-B
435 radiation on hydrated bdelloid *Philodina roseola* was almost complete at 96h. Moreover, in
436 this latter study they demonstrated that UV-B damage was only repaired when *P. roseola*
437 went through a desiccated state. Desiccation therefore appears a key factor to induce DNA
438 repair in bdelloids (Fischer et al., 2013).

439 How desiccated animals avoid apoptotic or necrotic processes that should normally be
440 induced by multiple DNA DSBs remains unknown and how organisms with a genome
441 shattered into small pieces can reassemble fully functional chromosome(s) also remains an
442 open question. When it comes to *A. vaga* an interesting hypothesis regarding repair is coming
443 directly from the genome structure described in Flot et al. (2013): the observed degenerate
444 tetraploidy could promote efficient DNA repair since homologous regions are useful for
445 template-dependent repair of DSBs as well as insure that, within the reservoir of gene copies,
446 at least one will remain intact after desiccation. The presence of intact copies of essential
447 genes may explain why during the recovery stage most individuals appear fully motile.

448 *UV-A and UV-B are modulating genomic integrity of desiccated A.vaga*

449 It has been demonstrated that environmental conditions such as temperature, relative humidity
450 or oxygen have an impact on the genome integrity of dried organisms (for instance on
451 bacteria, Yang et al., 2009) and on DNA (Bonnet et al., 2010). Recently, Hall et al. (2014)
452 studied the impact of environmental UV radiation on dried DNA and found that DNA DSBs
453 and oxidative lesions are the main damages induced.

454 In the present study, we tested the impact of UV-A and UV-B radiation on one-day desiccated
455 *A. vaga* and show that both UV types promote DNA DSBs (Fig. 4). Corroborating with our
456 results, UV-A and UV-B were also recently reported as sources of DNA DSBs in forensic
457 samples (Hall et al., 2014) or desiccated *Bacillus subtilis* (Moeller et al., 2007). We also

458 found that UV-A and UV-B induced damages were significantly affecting the survival rate of
459 desiccated *A. vaga* individuals in contrast with protonic radiation induced damages (Fig. 1C,
460 1D). The resulting decrease in survival rate may be linked to non-reversible oxidative damage
461 affecting key proteins involved in metabolism restoration since UV radiation also induces
462 considerable oxidative lesions (Altiero et al., 2011). In their natural environments, such as
463 mosses and lichens, *A. vaga* individuals experiencing desiccation are likely to be exposed to
464 solar UV radiations. In some specific conditions, *A. vaga* can be exposed to massive UV
465 irradiation, for instance in Arctic or Alpine mosses (Kaya et al., 2010; Fontaneto & Melone,
466 2003), or when carried by wind at high altitudes (under the form of contracted “tun”; Wilson
467 & Sherman 2013). Besides such extreme conditions, it is interesting to outline that the
468 relatively mild UV-A dose used in this study was able to significantly impair *A. vaga* survival
469 during desiccation. The dose used here appears in the same range of those experienced by
470 wild bdelloid populations exposed directly to sunlight in Belgium: on October 1st 2013 we
471 recorded doses of 45 kJ/m² UV-B and 200 kJ/m² UV-A during 7h direct exposure to sunlight.
472 Therefore, current results suggest that solar radiation might play an important role in
473 modulating the genomic integrity of desiccated bdelloids and their survival; since bdelloid
474 rotifers are asexual, one individual is sufficient to start a new population. A predictable
475 outcome is that important bottleneck events may reshape continuously *A. vaga* populations,
476 and together with desiccation may contribute to rapid speciation events in this clade.

477 *Evolutionary consequences of desiccation on an ameiotic evolution*

478 Living in temporary habitats, *A. vaga* individuals probably undergo multiple cycles of
479 desiccation during their life cycle. It has been hypothesized that desiccation-induced DNA
480 DSBs shape bdelloid genomes and promote gene conversion through mitotic recombination
481 during DNA DSB repair (Flot et al., 2013). The desiccation process also appears to be a key
482 mechanism to repair DNA damage, as it was observed in hydrated *P. roseola* individuals

483 where DNA damage induced by UV-B was only efficiently repaired after a round of
484 desiccation (Fischer et al., 2013). The interplay between the genome structure and DNA
485 repair of DSBs following desiccation events remains to be deciphered. Interestingly, a
486 degenerated Spo11 gene has been characterized in the genome of *A. vaga* although
487 conventional meiosis is not expected, and alternative roles of proteins usually involved in
488 meiotic pathways may also be interesting to investigate (Hörandl & Hadace 2013). Finally,
489 repetition of desiccation events over time associated with frequent DSBs may favor the
490 integration of horizontally transferred genetic material, accounting for 8% of the gene content
491 of *A. vaga* that appears to be of non-metazoan origin (Flot et al., 2013). This challenging but
492 exciting hypothesis remains to be tested, but if true would provide a remarkable example of
493 genomic adaptation to a natural environment as has been observed in plant-parasitic
494 nematodes (Paganini et al., 2012).

495 The clustering of *A. vaga* individuals during the dehydration process was positively linked
496 with survival rate. In nature, *A. vaga* individuals regroup before entering into desiccation and
497 are therefore more likely to increase genetic exchange between them. We have currently no
498 empirical data supporting genetic exchange between bdelloids. However, it seems likely that,
499 given the fact that the *A. vaga* genome contains 8% of horizontally acquired genes from non-
500 metazoans, exchange among bdelloids occur. The formation of DNA DSBs in desiccated
501 bdelloids is a plausible gateway to asexuality by promoting integration of horizontally
502 transferred genetic material during drought and rehydration cycles.

503

504 **Acknowledgments**

505 The authors are grateful to J.-F. Flot and L.-M. Pigneur (UNamur) for useful discussions and
506 comments. Thanks also to F. Chainiaux (UNamur) for allowing access to her UV

507 experimental equipment. J.N. Duprez and J. Mainil (Ulg) are highly acknowledged for their
508 technical help during the initiation phase of this project. Special thanks are attributed to C.
509 Laurent, J. Virgo and R. Tonneau (UNamur) for their precious technical support. The authors
510 are also grateful to D. Bonifazi (UNamur) for allowing access to chemistry department and
511 his technical support. This work was supported by a start-up grant from the University of
512 Namur to KVD.

513

514 **Author Contributions**

515 B.H., M.K. and D.H.M. maintained the rotifer cultures. B.H. performed all experiments with
516 help of M.K. for the group effect study and D.H.M. and M.K. contributed to the optimization
517 of the desiccation protocol and PFGE. A-C.H. and B.H. performed the proton exposures,
518 supervised by S.L. TGA were performed by F. P and B.H. The results were analyzed and the
519 manuscript written by B.H., R.K. and K.V.D. The project was designed and supervised by
520 R.K. and K.V.D. and the acquisition of funding was done by K.V.D.

521

522 **FIGURE LEGENDS**

523 **Figure 1. Effect of group, time and radiation on the survival rate of desiccated *Adineta***
524 ***vaga* individuals. (a)** Survival rate of *Adineta vaga* as a function of the number of individuals
525 during 14 days of desiccation (Material and Methods) **(b)** Effect of time spent in desiccated
526 state on the survival rate of *A. vaga* 24h after rehydration. **(c)**. Effect of proton radiation on
527 the survival rate of *A. vaga* who spent one day in desiccated state (Material and Methods). **(d)**
528 Effect of UV-A (diamonds) and UV-B (squares) radiation on the survival rate of *A. vaga* who
529 spent one day in desiccated state. Standard deviations are represented by error bars.

530 **Figure 2. Genome integrity of desiccated *Adineta vaga*.** The three panels show PFGE. The
531 first and last lanes on the PFGE correspond to the karyotype of *Saccharomyces cerevisiae*
532 without and with treatment to lysis buffer. Other lanes correspond to 1,000 *A. vaga*
533 individuals submitted to desiccation and/or radiation. The control on each gel corresponds to
534 1,000 hydrated *A. vaga* individuals. **(a)** Effect of time (1, 7, 14, 21, 42 and 84 days) spent in
535 desiccated conditions on the apparition of DNA DSBs. **(b)** Apparition of DNA DSBs during
536 the time spent in dry conditions (1, 21 or 42 days) in living (d+) and dead (d-) animals. **(c)**
537 Effect of proton irradiation (in gray Gy) on the apparition of DNA DSBs in one-day
538 desiccated animals.

539 **Figure 3. (a).** Repair kinetic of rehydrated *A. vaga* after 21 days of desiccation. **(b).** Repair
540 kinetic of rehydrated *A. vaga* after one day of desiccation with exposure to 800 Gy proton
541 radiation. The first and last lanes on the PFGE correspond to the karyotype of *S. cerevisiae*
542 without and with treatment with lysis buffer. Second lanes correspond respectively to 21 days
543 desiccated bdelloids and one day desiccated bdelloids submitted to 800 Gy proton radiation.
544 Other lanes correspond to 1,000 desiccated *A. vaga* individuals after 2, 4, 8, 24 and 48 hours
545 of rehydration.

546 **Figure 4.** PFGE analysis of the effect of UV-A **(a)** and UV-B **(b)** on the genome integrity of
547 one-day desiccated *A. vaga* individuals. The first lane on the PFGE corresponds to the
548 karyotype of *S. cerevisiae*. Other lanes correspond to 1,000 *A. vaga* individuals desiccated for
549 one day and exposed to UV for 50, 100 or 150KJ. For each irradiation experiment, 1,000 *A.*
550 *vaga* individuals were exposed to the same temperature (Ctl t°) for the same amount of time
551 presented.

552

553

554 **References**

- 555 Alpert, P. 2006. Constraints of tolerance: why are desiccation-tolerant organisms so small or
556 rare? *J. of Exp. Biol.*, 209: 1575-1584.
- 557 Altiero, T., Guidetti, R., Caselli, V., Cesari, M., Rebecchi, L. 2011. Ultraviolet radiation
558 tolerance in hydrated and desiccated eutardigrades. *J Zool Syst Evol Res*, 49,1439-0469
- 559 Billi., D. 2009. Subcellular integrities in Chroococciopsis sp. CCMEE 029 survivors after
560 prolonged desiccation revealed by molecular probes and genome stability assays.
561 *Extremophiles* 13:49–57.
- 562 Boschetti, C., Carr, A., Crisp, A., Eyres, I., Wang-Koh, Y., (...), Tunnacliffe, A . 2012.
563 Biochemical diversification through foreign gene expression in bdelloid rotifers. *PLOS Genet*
564 8: e1003035.
- 565 Bonnet J, Colotte M, Coudy D, Couallier V, Portier J, Morin B, Tuffet S. 2010. Chain and
566 conformation stability of solid-state DNA: implications for room temperature storage. *Nucleic*
567 *Acids Res.* 38(5):1531-46.
- 568 Danchin E.G.J., Flot J.-F., Perfus-Barbeoch L., Van Doninck K. 2011. Genomic perspectives
569 on the long-term absence of sexual reproduction in animals. In: *Pontarotti P (ed.) Evol*
570 *Biology – Concepts, Biodiversity, Macroevol and Gen Evol.* Springer, Berlin Heidelberg (pp
571 223-242).
- 572 Davila J. I, Arrieta-Montiel M. P, Wamboldt Y, Cao J, Hagmann J, et al. 2011. Double-strand
573 break repair processes drive evolution of the mitochondrial genome in *Arabidopsis*. *BMC*
574 *Biology* 9: 64.

575 Davis, H. 1873. A new Callidina: with the result of experiments on the desiccation of rotifers.
576 *Month. Microscopical J.* 9, 201–209.

577 Fischer, C., Ahlrichs, W., Buma, A., van de Poll, W., Bininda-Emonds, O. 2013. How does
578 the ‘ancient’ asexual *Philodina roseola* (Rotifera: Bdelloidea) handle potential UV-B-induced
579 mutations? *The J. of Exp. Biol.* 216, 3090-3095.

580 Flot, J.-F., Hespels, B., Li, X., Noel B. (34 authors), Jaillon, O. & Van Doninck, K. 2013.
581 Genomic evidence for ameiotic evolution in the bdelloid rotifer *Adineta vaga*. *Nature*,
582 500:453–457.

583 Fontaneto, D. & Melone, G. 2003. Bdelloid Rotifers from Lakes above 1700 m in Western
584 Italian Alps, with Taxonomic Notes on *Dissotrocha macrostyla*. *Int Rev of Hydrobiol* 88:
585 594–601

586 França, M.B., Panek, A.D., Eleutherio, E.C.A. 2007. Oxidative stress and its effects during
587 dehydration. *Comp. Biochem. Physiol. A Comp. Physiol.* 146, 621–631.

588 Gladyshev, E., Meselson, M. 2008. Extreme resistance of bdelloid rotifers to ionizing
589 radiation. *Proc Natl Acad Sci U S A* 105: 5139–5144.

590 Gladyshev, E. and Arkhipova, I. 2010. Genome Structure of Bdelloid Rotifers: Shaped by
591 Asexuality or Desiccation? *J. of Hered.* 2010:101(Supplement 1):S85–S93.

592 Gusev, O., Nakahara, Y., Vanyagina, V., Malutina, L., Cornette, R., (6 authors), Okuda, T.
593 2010. Anhydrobiosis-associated nuclear DNA damage and repair in the sleeping chironomid:
594 linkage with radioresistance. *PLoS ONE* 5(11): e14008.

595 Hall, A., Sims, LM., Ballantyne, J. 2014. Assessment of DNA damage induced by terrestrial
596 UV irradiation of dried bloodstains: Forensic implications. *Forensic Sci. Int.: Genetics* 8. 24–
597 32.

598 Hörandl, E., Hadacek, F. 2013. The oxidative damage initiation hypothesis for meiosis. *Plant*
599 *Reprod.*

600 Hsu, W. S. 1956. Oogenesis in *Habrotrocha tridens* (milne). *Biol. Bull.*, Vol. 111 pp. 364–
601 374.

602 Hsu, W. S. 1956. Oogenesis in the Bdelloidea rotifer *Philodina roseola* Ehrenberg. *Cellule*
603 57, 283–296

604 Ivarsson, H., Jönsson, I. 2004. Aggregation Effects on anhydrobiotic survival in the tardigrade
605 *Richtersius coronifer*. *J. of exp. zool.* 301A:195–199.

606 Kaya, M., De Smet, W., Fontaneto, D. 2010. Survey of moss-dwelling bdelloid rotifers from
607 middle Arctic Spitsbergen (Svalbard). *Polar Biology*, Volume 33, Issue 6, pp 833-842.

608 Krisko, A., Leroy, M., Radman, M. & Meselson, M. 2012. Extreme anti-oxidant protection
609 against ionizing radiation in bdelloid rotifers. *Proc. Natl Acad. Sci. USA* 109, 2354–2357.

610 Mark Welch, DB., Meselson, M. 2000. Evidence for the evolution of bdelloid rotifers without
611 sexual reproduction or genetic exchange. *Science* 288: 1211–1215.

612 Mark Welch, JL., Meselson, M. 1998. Karyotypes of bdelloid rotifers from three families.
613 *Hydrobiologia* 387/388: 403–407.

614 Marotta R, Uggetti A, Ricci C, Leasi F, Melone G. (2012). Surviving starvation: Changes
615 accompanying starvation tolerance in a bdelloid rotifer. *J Morphol* 273: 1-7.

616 Mattimore, V. and Battista, JR. 1996. Radioresistance of *Deinococcus radiodurans*: functions
617 necessary to survive ionizing radiation are also necessary to survive prolonged desiccation. *J.*
618 *Bacteriol.* 178(3):633-637.

619 Moeller, R., Stackebrandt, E., Reitz, G., Berger, T., (3 authors), Horneck, G. and Nicholson,
620 W. L. 2007. Role of DNA repair by non-homologous end joining (NHEJ) in *Bacillus subtilis*
621 spore resistance to extreme dryness, mono- and polychromatic UV and ionizing radiation. *J.*
622 *Bacteriol.* 189:3306-3311.

623 Neumann, S., Reuner, A., Brümmer, F., O. Schill, R. 2009. DNA damage in storage cells of
624 anhydrobiotic tardigrades. *Comp. Bioch. and Physiol., Part A* 153 425–429.

625 Pace, J. K 2nd., Sen, SK., Batzer, M. A., Feschotte, C. 2009. Repair-mediated duplication by
626 capture of proximal chromosomal DNA has shaped vertebrate genome evolution. *PLoS Genet*
627 5: e1000469.

628 Paganini, J., Campan-Fournier, A., Da Rocha, M., Gouret, P., Pontarotti, P., Wajnberg, E.,
629 Abad, P. and Danchin, E.G.J. 2012. Contribution of lateral gene transfers to the genome
630 composition and parasitic ability of root-knot nematodes. *PLoS One*, 7, e50875

631 Ricci, H., Caprioli, C., and Fontaneto, D. 2007. Stress and fitness in parthenogens: is
632 dormancy a key feature for bdelloid rotifers? *BMC Evol Biol*, 7(Suppl 2):S9.

633 Segers, H. 2007. Annotated checklist of the rotifers (Phylum Rotifera), with notes on
634 nomenclature, taxonomy and distribution. *Zootaxa*. 1564, 1–104.

635 Shirkey, B., McMaster, NJ., Smith, SC., Wright, DJ., Rodriguez, H., Jaruga, P., Birincioglu,
636 M., Helm, RF., Potts, M. 2003. Genomic DNA of *Nostoc commune* (Cyanobacteria) becomes
637 covalently modified during long-term (decades) desiccation but is protected from oxidative
638 damage and degradation. *Nucl. Acids Res*, 31:2995-3005

639 Van Doninck, K., Mandigo, ML., Hur, JH., Wang, P., Guglielmini,. (2 authors), Meselson, M.
640 2009. Phylogenomics of unusual histone H2A Variants in Bdelloid rotifers. *PLoS Genet.*

641 Wera, A-C., Donato, K., Michiels, C. , Jongen, Y. & Lucas, S. 2008. Preliminary results of
642 proton beam characterization for a facility of broad beam in vitro cell irradiation. *Nucl. Inst.*
643 *and Meth. in Phys. Res. B* . 266 , 10 , p. 2122-2124 , 3 p.

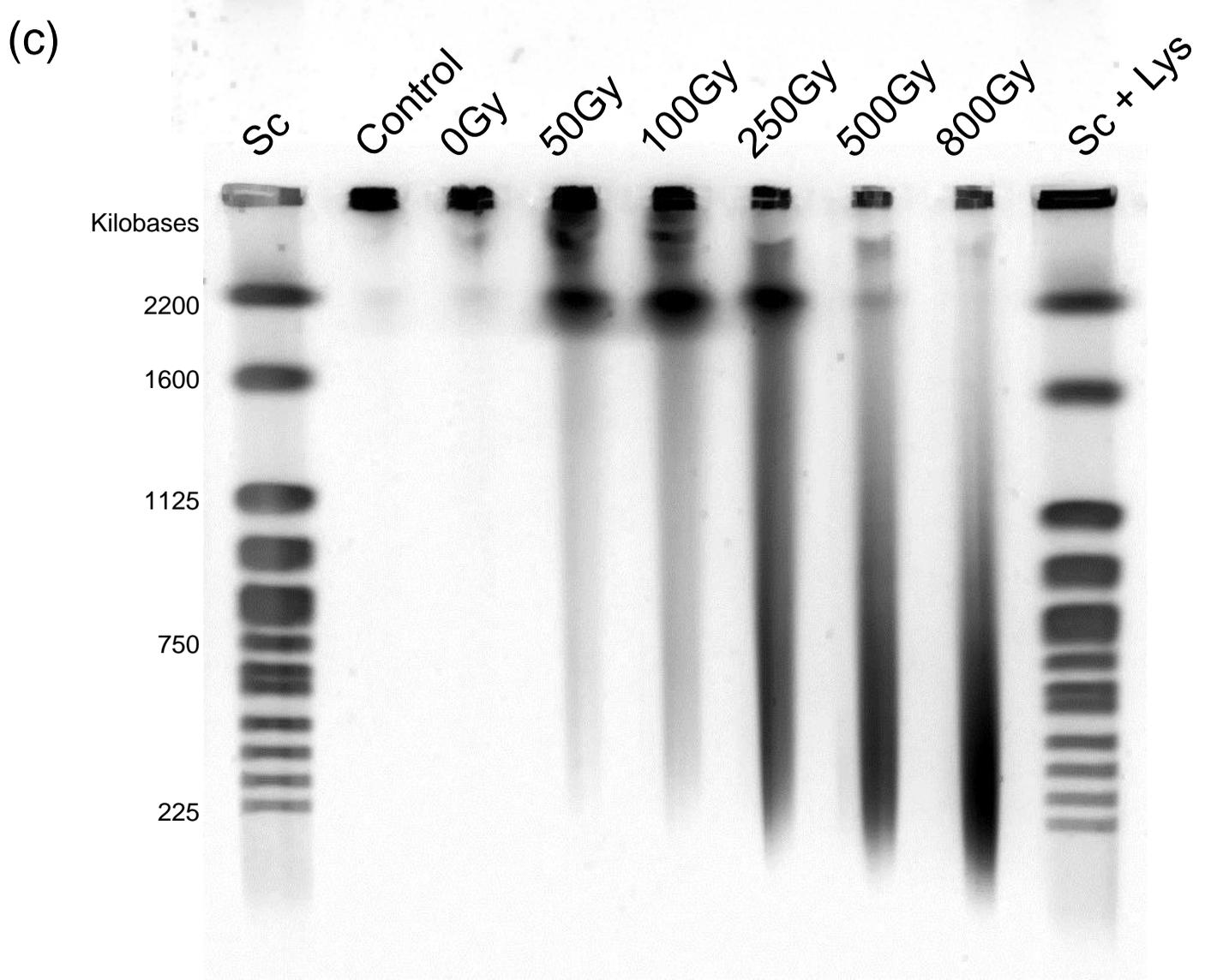
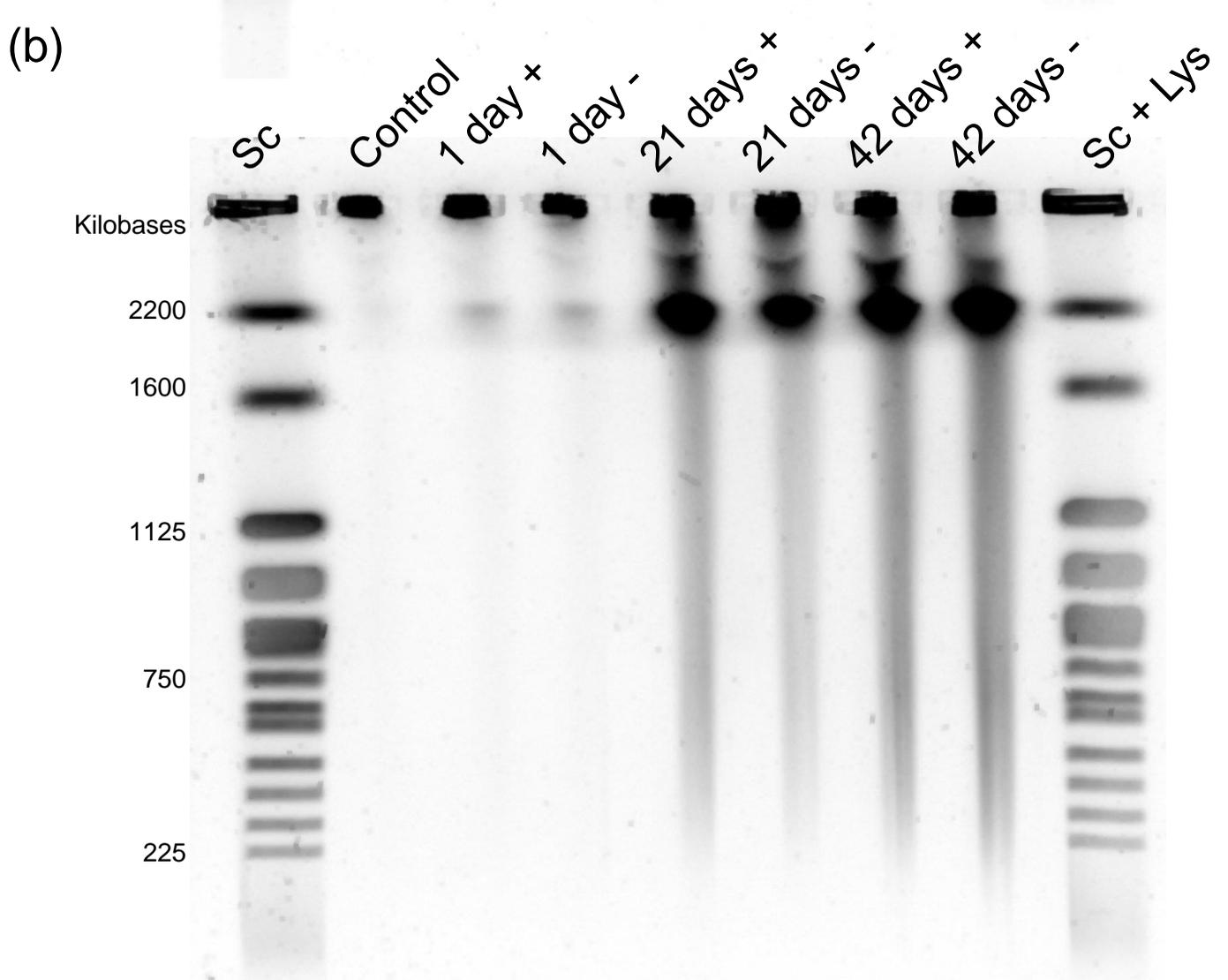
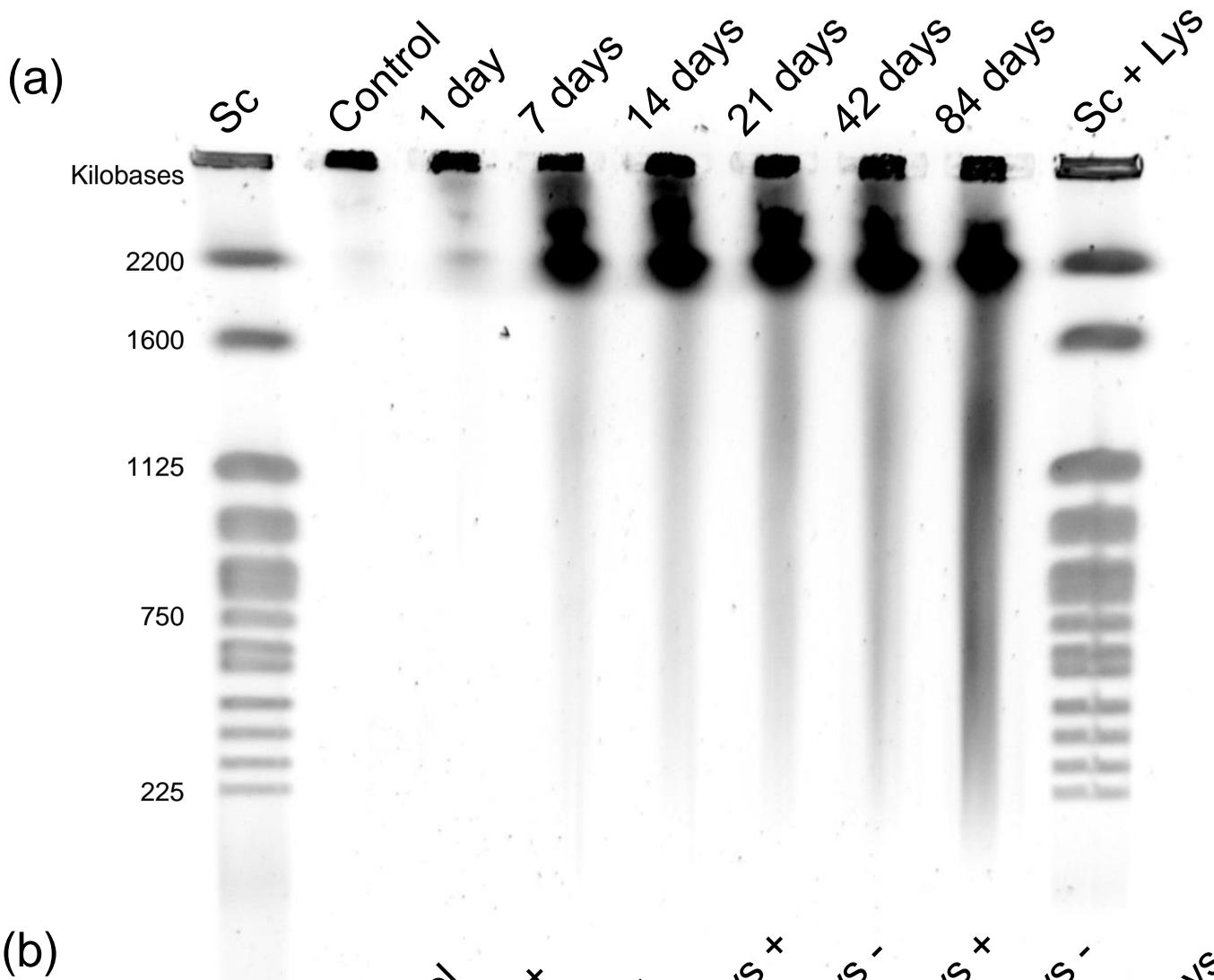
644 Wera, A-C. , Heuskin, A-C. , Riquier, H. , Michiels, C. & Lucas, S. 2011. In vitro irradiation
645 station for broad beam radiobiological experiments. *Nucl. Inst. and Meth. in Phys. Res B* .
646 269, 24 , p. 3120-3124 , 5 p.

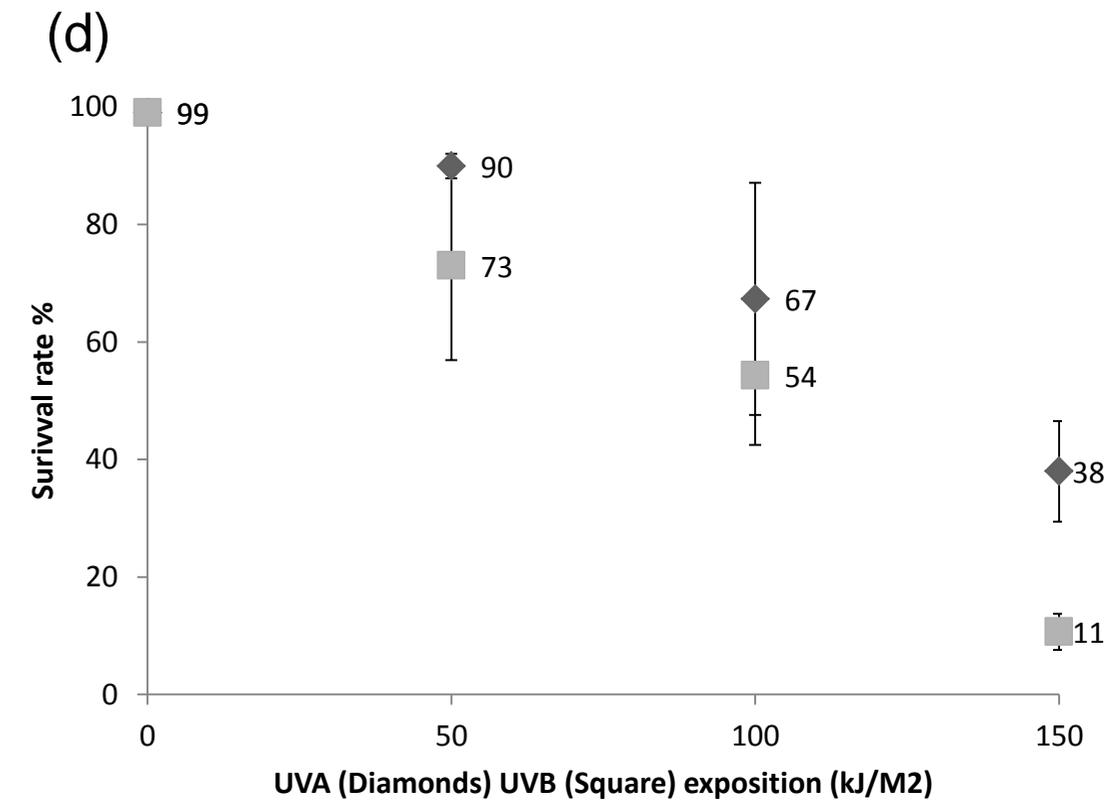
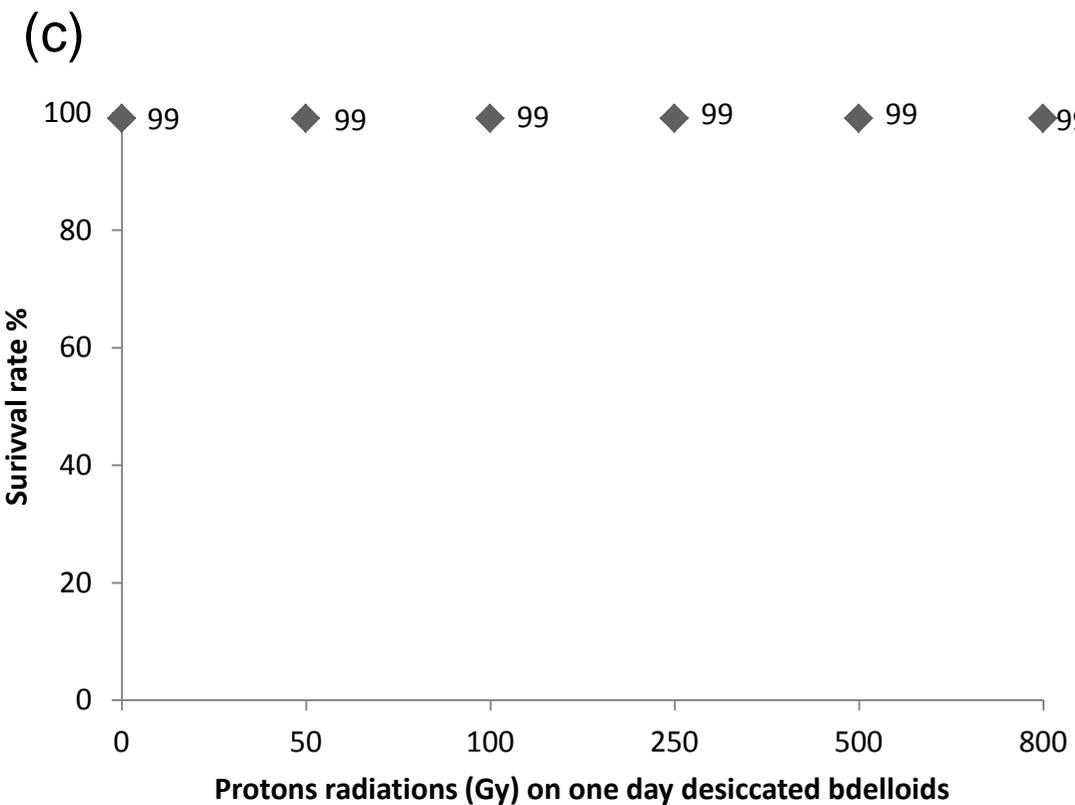
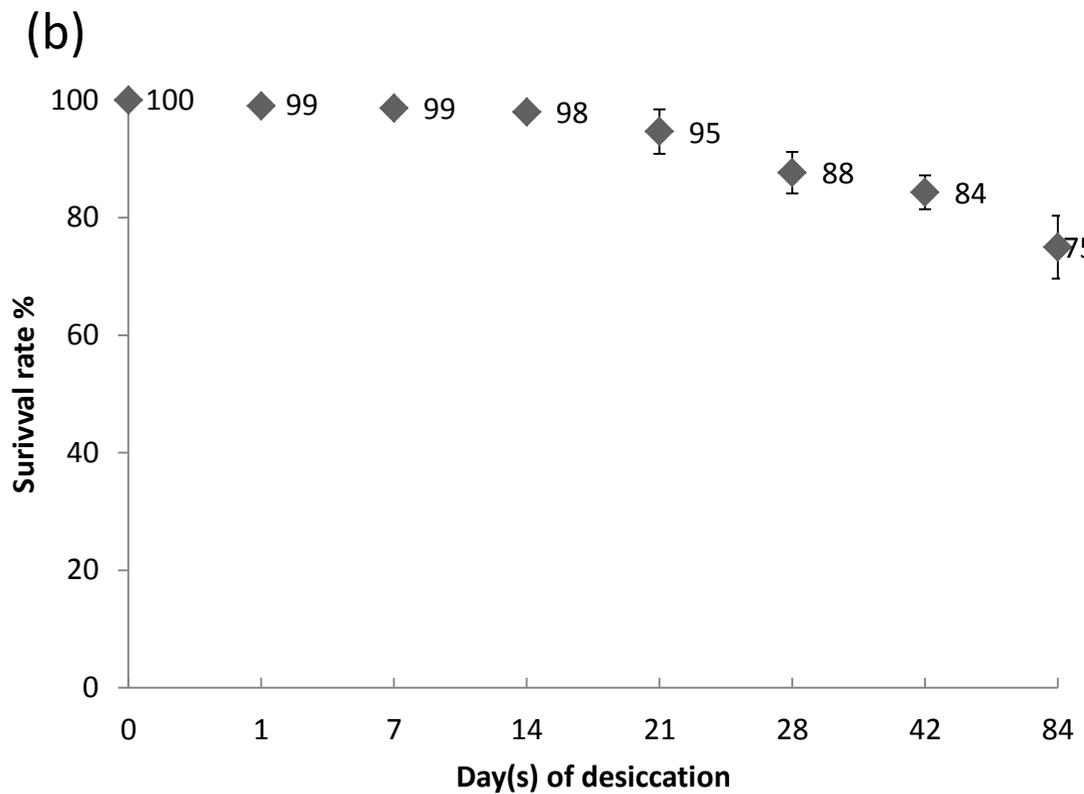
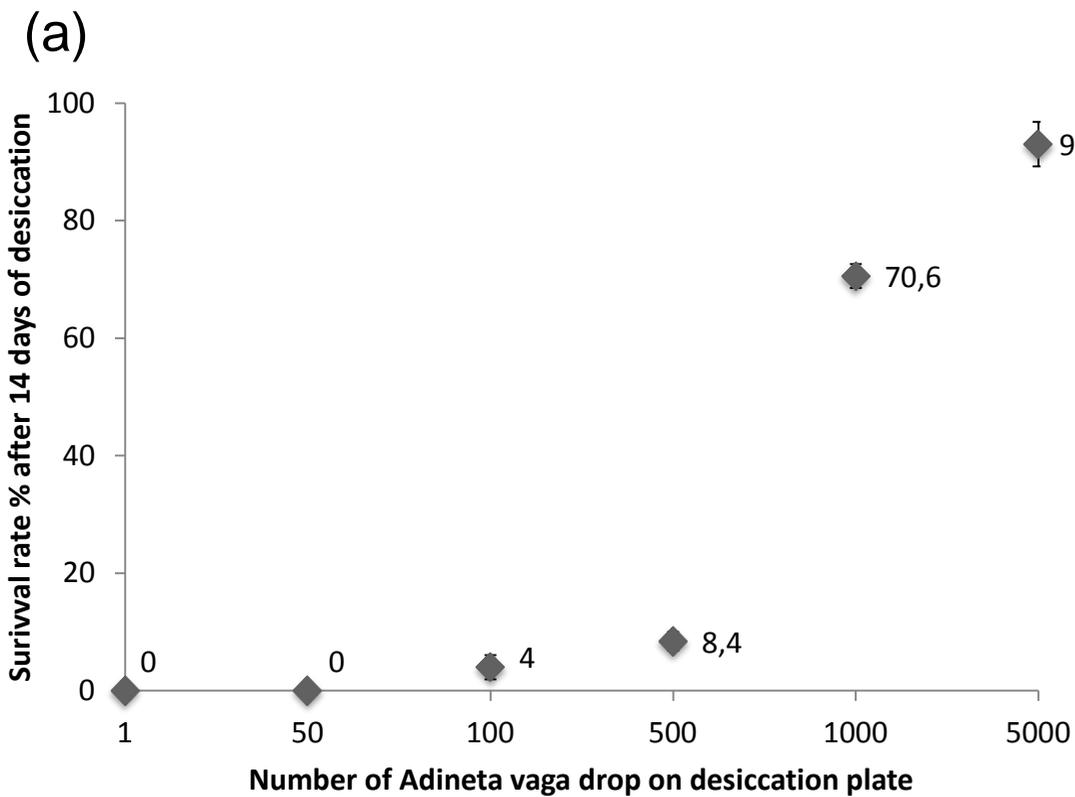
647 Wilson, CG., Sherman, PW. 2013. Spatial and temporal escape from fungal parasitism in
648 natural communities of anciently asexual bdelloid rotifers. *Proc Biol Sci.* Jul
649 3;280(1765):20131255.

650 Yang, Y., Yokobori, S., Yamagishi., A. 2009. Bacterial survival in response to desiccation
651 and high humidity at above zero and subzero temperatures. *Adv. in Sp. Res.* 43 1285–1290.

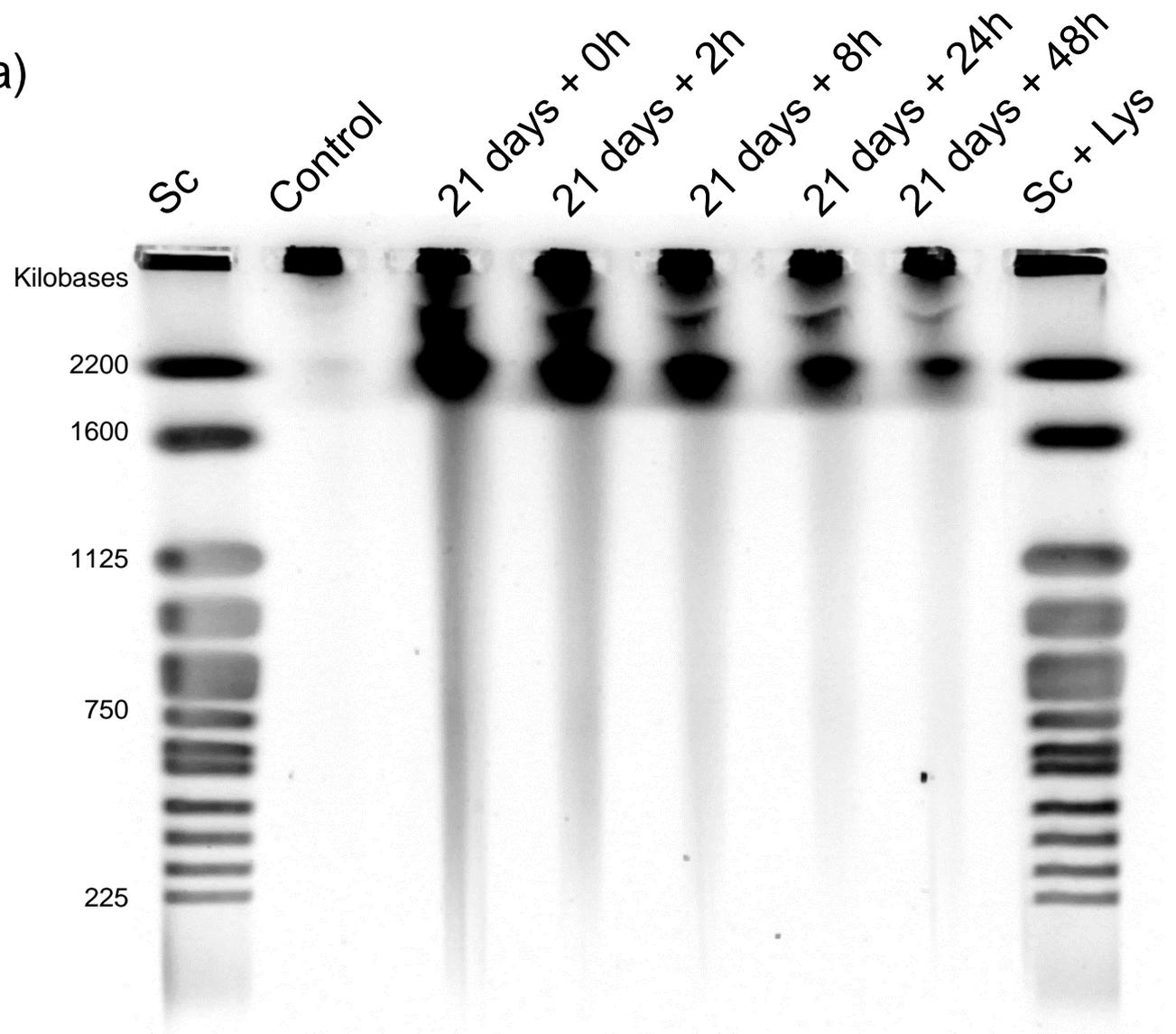
652 Zahradka, K., , Slade, D., Bailone, A., Sommer, S., Averbek, D., Petranovic, M., Lindner
653 A.B., Radman, M. 2006. Reassembly of shattered chromosomes in *Deinococcus radiodurans*.
654 *Nature*, 443, 569-573

655 Zimmerman JM, Battista JR. (2005). A ring-like nucleoid is not necessary for radioresistance
656 in the Deinococcaceae. *BMC Microbiol* 5: 17.

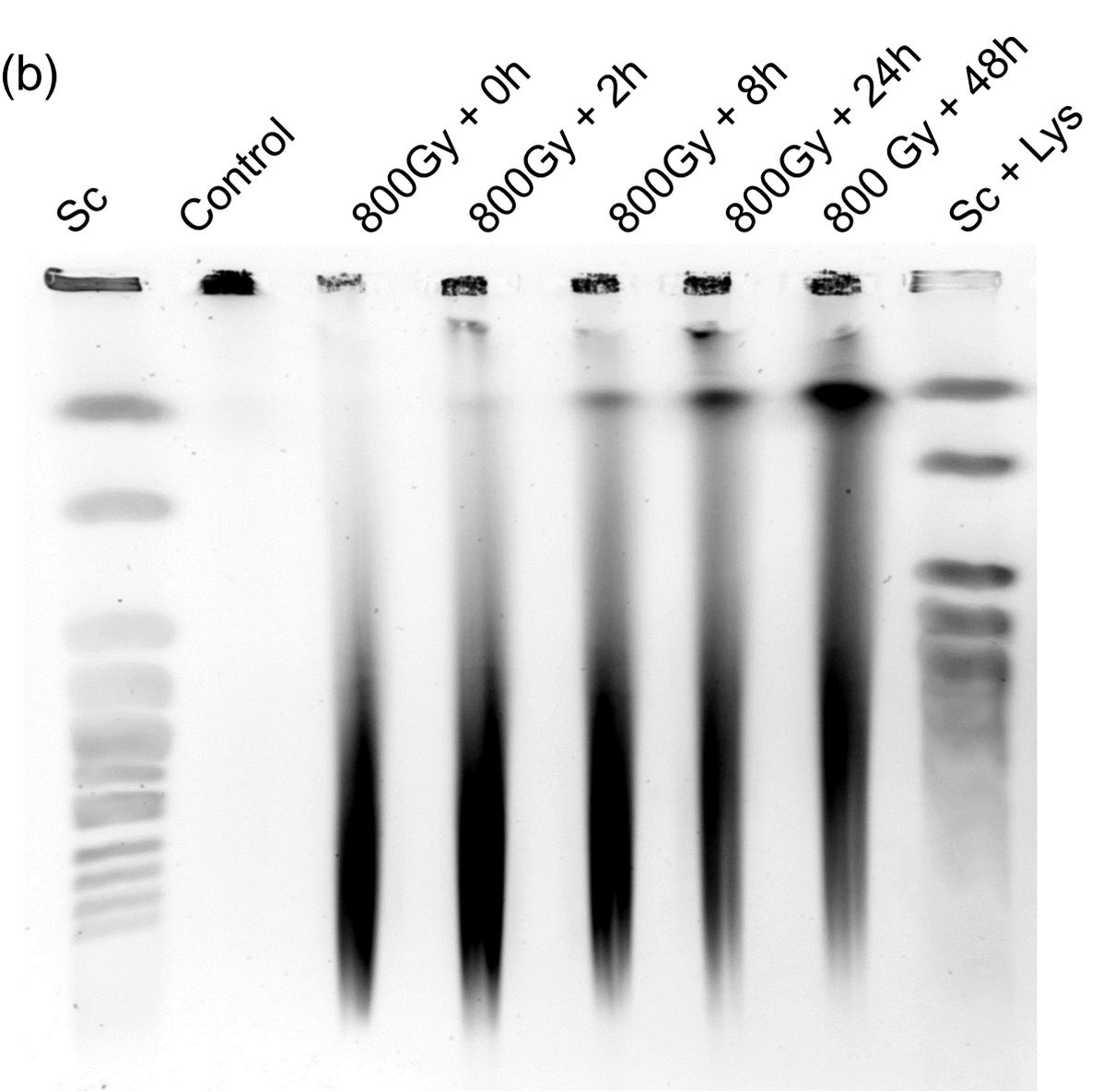




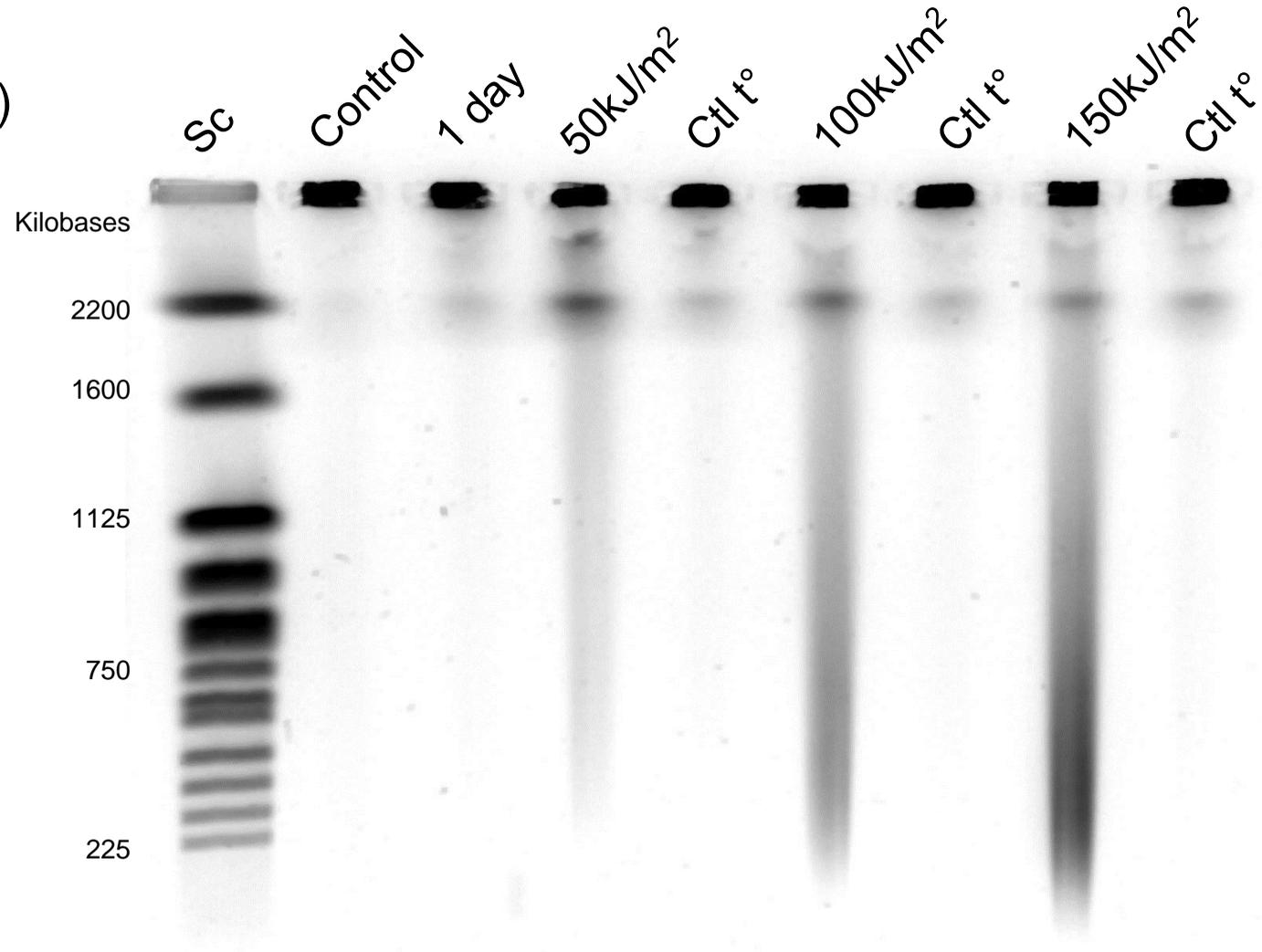
(a)



(b)



(a)



(b)

